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SURFACE POLYMERIZATION OF POLY(γ-ALKYL-L-GLUTAMATE) ON SOLID SUBSTRATES

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Abstract: Surface polymerization of poly(γ-methyl-L-glutamate) and a copolymer of γ-methyl and γ-stearyl-L-glutamate are conducted on aminopropyl triethoxysilane modified silicon(100) native oxide substrates. The film formation is monitored by X-ray photoelectron spectroscopy, and the conformation and orientation of surface-bound polypeptides are characterized by transmission Fourier transform infrared spectroscopy. Both films are formed as β-sheet material, and the film orientations are tilted from the solid substrate.

INTRODUCTION

Synthetic polypeptides, especially polyglutamates (PG) and their copolymers, have been widely studied for their liquid crystalline, amphiphilic properties[1]. It has been shown that PGs are excellent materials for the formation of Langmuir-Blodgett films[2,3,4]. When such films are transferred from the air-water interface onto hydrophilic solid substrates, the films are physically adsorbed with the chain axes oriented parallel to the substrate. By contrast, self-assembled adsorption, a process in which films are chemically adsorbed onto the solid substrate as well as being aligned with a preferential orientation, suggests an alternate approach to fabricate thin films with the molecular axes perpendicular to the substrate[5]. Such a configuration may be of interest for fabrication of anisotropic membranes, liquid crystal displays, or sensors. Because of these potential applications, many researchers have attempted to fabricate such films on solid substrates by a (grafting *to*) self-assembly process[6,7,8]. However, all these studies have encountered difficulties in fabricating high molecular weight surface-bound materials, which is a direct result of the low

accessibility of high molecular weight polypeptides to the surface functional groups. Thus, the average film thicknesses are on the order of $10 \sim 10^2$ Å. Moreover, it has been suggested that the molecular weight of the grafted polypeptides should be less than 50 kd to prevent the physisorption resulting from non-specific binding of side chains to the solid substrate [7].

In order to construct a film with the high molecular weight polypeptides oriented perpendicular to the substrate, Whitesell et al. first took advantage of covalently attached amino trithiol groups on gold and methoxysilane groups on indium-tin-oxide glass surfaces, and used these amino groups as initiators to polymerize the N-carboxy anhydrides (NCAs) of alanines [9]. They claimed that their surface polyalanine has a perpendicular helical structure, with a film thickness of 1000 Å, measured by grazing angle FTIR and ellipsometry. Recently, Wieringa and Schouten spin-cast the NCA of γ-methyl-L-glutamate on a primary amine modified silicon wafer, and then heated the substrate above the NCA melting temperature to induce surface polymerization. The resulting film, after solvent wash had a helical structure and a thickness of 200 Å [10]. Similarly, Heise et al. also performed a surface polymerization of γ-benzyl-L-glutamate initiated by an amino terminated self assembled monolayer on a silicon wafer, with the surface density of the initiator diluted through use of binary mixtures. They discovered that the thickness of polymerized films is about 45 to 60 Å [11].

In contrast to these reports, we also have discussed poly(γ-methyl-L-glutamate) (PMLG) polymerized from the surface by a surface initiator amino group which is converted from the surface-bound 1-cyano-16-trichlorosilyl hexadecane; the resulting film contains β-sheet conformation with a preferential orientation more perpendicular to the substrate [12]. In order to fabricate a film with high molecular weight polypeptide perpendicular to the substrate, one speculation was that the initiator molecules need to have enough intermolecular space to allow a subsequent helical growth. In other words, a well-controlled low density initiator layer is the key for a successful surface polymerization. However, the results from both Heise and coworkers [11] and our group [12] show no significant correlation between film thickness or secondary structure and the surface density of the initiator layer. The main objective of this work is, therefore, to explore further the possible causes leading to different film structures through the surface polymerization (grafting *from*) approach. Instead of varying the surface density of the initiator, NCAs with different side chains from various glutamate derivatives are used. The two polyglutamate derivatives are PMLG and a copolymer of γ-methyl(70%) and γ-stearyl (30%)-L-glutamate) (PMSLG) with aminopropyl triethoxysilane (APS) as the initiator layer.

RESULTS AND DISCUSSION

The synthesis of monomer NCAs [13, 14] and the film preparation[6] are described elsewhere. The APS layer is first deposited on a freshly cleaned silicon (100) native oxide substrate, and film formation is confirmed by X-ray photoelectron spectroscopy (XPS) (appearance of N(1s) signal at 399.3 eV), contact angle measurement (advancing water contact angle of 39°-42°), and ellipsometry (8Å ± 1Å). The freshly prepared APS modified substrate is then immersed in the 0.1M NCA in anhydrous THF at room temperature for various time intervals from 20 to 60 hours. The substrate is then cleaned by chloroform, isopropanol, and gently wiped with a camel hair brush to remove the loosely adsorbed materials. The XPS measurement was conducted to obtain the experimental stoichiometric ratios of both films, and we found that the ratios are consistent with their theoretical values (for PMLG, C:N:O= 6:3:1; for PMSLG, C:N:O= 11:3:1) within experimental error.
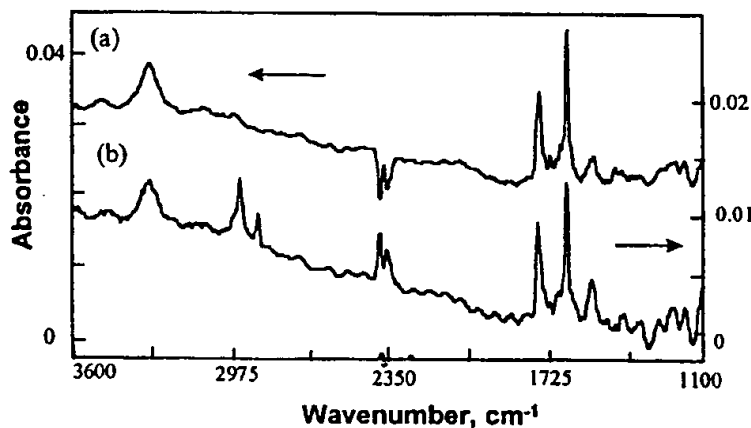

Figure 1. IR spectra of surface polyglutamates (a) PMLG (b) PMSLG

The secondary structure and the orientation of the films are further examined by transmission Fourier transform infrared spectroscopy (t-FTIR). The secondary structure of polypeptides can be identified by the peak locations of the amide I (C=O stretching) and the amide II (C-N stretching coupled with N-H bending) bands. For a typical α-helical conformation, the absorbance band of the amide I is at 1650~1654 cm^{-1} and the amide II is at 1548~1550 cm [-1],

respectively. For a β-sheet structure, the absorbance band of the amide I is at about 1625 cm^{-1} and the amide II is at about 1520 cm^{-1}. As shown on Figure 1, both grafted PMLG and PMSLG films have the amide I and the amide II at 1625 cm^{-1} and 1520 cm^{-1}, indicating a β-sheet conformation. The film orientation can be approximately calculated through the comparison of the ratio of the amide I or the amide II to the carbonyl ester stretch at 1730-1738 cm^{-1}. In a β-sheet structure, the dipole moment of the amide I band is perpendicular to the backbone axis, while the amide II band is parallel. By contrast, the vibration of the side chain carbonyl ester stretch is considered to be invariant to the backbone orientation. Table 1 is the comparison of the IR absorbance ratios from the surface PMLG, and the bulk synthesized β-sheet PMLG dispersed in a KBr pellet, in which we assume that the orientation of the molecular axes is randomly distributed. For the IR measurement with the EM field parallel to the solid substrate, the higher ratio of the amide I to the carbonyl stretch of the film sample (1.8) compared to that of the isotropic value of the bulk (0.9), and the lower ratio of the amide II to the carbonyl ester stretch of the film sample (0.3) compared to that of the bulk (0.6) indicates that the vibration of the surface amide I is more parallel while the surface amide II is more perpendicular to the EM field. Thus, the overall molecular axis is likely to be more perpendicular to the solid substrate. The same conclusion can be drawn by the comparison of the ratio of the amides I and II to the carbonyl ester stretch for the surface PMSLG sample, as shown in Table 1(c).

	I amide I / Iυ c=o ester	I amide II / Iυ c=o ester
(a) PMLG dispersed in KBr pellet(β-sheet)	0.9	0.6
(b) PMLG film (β-sheet) (EM vector //substrate)	1.8	0.3
(c) PMSLG film (β-sheet) (EM vector //substrate)	1.4	0.4

Table 1. Orientation study of surface PGs by comparison of the IR absorbance ratios of I amide I /I c=o ester and I amide II / I c=o ester for both bulk and surface PGs .

β-sheet PMLG in both (a) bulk (KBr pellet) and (b) surface; (c) β-sheet PMSLG on surface. The absorbance of the amide I=1626 cm^{-1}, amide II=1526 cm^{-1}, and υ c=o ester =1737 cm^{-1}, respectively.

In this study, we found that both PMLG and PMSLG can be polymerized from the surface by an APS initiator layer, although both polypeptides have much larger cross sectional area than

that of an APS molecule; this observation indicates an insensitivity to the relative size difference between the grafted polypeptide and the initiator molecule. However, according to the IR absorbances, the film thickness of PMLG is approximately 1.5 times the thickness of PMSLG, which suggests that the hindrance effect is more pronounced in a larger polypeptide. In contrast to an α-helical structure of PMLG obtained by Wieringa et al. [10], both surface-initiated PMLG and PMSLG favor a β-sheet formation in our systems. It has been shown that the NCA monomer synthesized by the standard work-up procedure can contain a substantial amount of the corresponding glutamate hydrochloride [15]. Dorman and his coworkers removed the byproduct by rephosgenation during the work-up procedure, and they found that using the ultra-pure NCA in the solution polymerization can greatly improve the resulting molecular weight of the polyglutamates[16]. Therefore, we speculate that the existence of the glutamate hydrochloride in our NCA compounds is the cause of an early termination of the surface polymerization[17], thus only low molecular weight oligomers of β-sheet PMLG and PMSLG are formed.

SUMMARY

In this work, we have demonstrated the surface polymerization of PMLG and PMSLG on the APS modified silicon substrates. We found that the resulting film structure, such as the secondary conformation and orientation, is greatly affected by the purity of the monomers and the length of the side chains. Therefore, in contrast to the grafting to approach in which the secondary conformation and the molecular weight of the grafted polypeptides can be controlled beforehand, one needs to consider these factors in order to successfully prepare the desired films by the grafting from method.

ACKNOWLEDGEMENT

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